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Effect of C/N ratio levels and stocking density of *Labeo victorianus* on pond environmental quality using maize flour as a carbon source

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ABSTRACT

The main obstacle in developing intensive fish culture is waste management which is detrimental to the environment. To mitigate environmental impacts associated with effluent discharge, measures should be put in place to avoid deterioration of the pond environment. The addition of carbon materials into culture facilities while manipulating the levels of carbon and nitrogen ratios is one of the best strategies of controlling ammonia and nitrite in ponds. This study was carried out in 18 hapas suspended in six, 150 m^2 earthen ponds to investigate the effects of C/N ratios (10 and 20) and stocking density (10, 15 and 25 fish m^{-2}) on water quality, sediment quality and growth of *Labeo victorianus*. All treatments were carried out in triplicate during a time period of 72 days. A locally formulated and prepared feed containing 30% crude protein with a C/N ratio of 10 was applied. Maize flour was used as the carbohydrate source for manipulating C/N ratio and applied to the water column separately from the feed. Increasing C/N ratio from 10 to 20 reduced ($P < 0.001$) the total ammonia nitrogen (TAN), nitrite–nitrogen (NO_2–N) and nitrate–nitrogen (NO_3–N) in the water column and total nitrogen in the sediment ($P < 0.001$). It also raised sediment pH, organic matter and total phosphorus ($P < 0.001$). The lowest protein efficiency ratio (PER), specific growth rate (SGR) and the highest food conversion ratio for the feed were recorded with a C/N ratio of 10 ($P < 0.05$). Based on highest growth, survival, production and net benefits, C/N ratio of 20 and a stocking density of 25 fish m^{-2} are optimal. Therefore, carbohydrate addition in *L. victorianus* culture is a promising option for sustainable aquaculture.

1. Introduction

As the aquaculture industry in East African region develops towards intensification and higher productivity, the demand for efficient culture systems and low cost of production is becoming crucial. Heightened intensification, which comes hand in hand with increase in culture densities and higher input levels, inevitably leads to greater environmental impacts. Appropriate stocking densities in intensive and semi intensive culture systems help in maintaining water quality balances which are vital for profitable pond harvests. In most cases the principal factor affecting water quality is the amount of feed supplied and subsequent release of metabolites in form of nutrients (Milstein, 1990). Good husbandry practices like stocking densities, nutrient ratios, aeration and water exchange are aimed at reducing the impacts of metabolites that would hamper water quality. Finding a right balance between nutrient ratios and carrying capacity in fish ponds while maintaining favorable culture conditions is a common problem in aquaculture pond management. Discharges from aquaculture deteriorate the receiving environment with fish serving as reservoirs of nutrients generated from the culture environment. Fish may also transport nutrients between compartments within an ecosystem or transfer nutrients to other ecosystems (Tanner et al., 2000). Different measures have been used to remove nitrogen from water: use of biological filters, addition of substrate for periphyton development, use of bio-floc technology and C/N ratio control (Avnimelech, 2007).

Stocking density is a major factor affecting fish survival, growth, behavior, health, water quality, feeding and production (Backiel and LeCren, 1978; Rui et al., 2006). For the development of rearing techniques, appropriate stocking densities must be determined for each species passing through successive production stages to enable efficient management and maximize production benefits. Culture of fish in enclosures applying the right stocking density can effectively improve the yield per unit area. However, stocking density can also cause stress, lower the feed conversion efficiency, increase fish energy consumption and reduce feeding rate and digestibility (Gibtan et al., 2008; Islam et al., 2006; Rowland et al., 2006; Wallat et al., 2004).

Labeo victorianus (Boulenger, 1901), commonly known as Ningu, is a potamodromus fresh water fish native of Lake Victoria (Greenwood,

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1966). The fish was once widely distributed in the Lake Victoria basin and supported the most important fishery of all the potamodromus species in the lake (Cadwalladr, 1965). The introduction of gill nets, set at the river mouths during spawning migrations, has been reported to be responsible for the rapid decline of the species since the 1950s (Ogutu-Ohwayo, 1990; Rutaisire, 2003; Seehausen, 1996). An alternative to ameliorate this problem is captive propagation. Although *L. victorianus* has not been cultured widely, it has potential where besides production of rich protein food, it would consequently reduce fishing pressure on the wild stocks. Members of the same genus rohu, *Labeo rohita* and *Labeo fimbriatus,* are widely cultivated in India (Halder et al., 1991; Jhingran and Pullin, 1985; Mridula et al., 2003) and *Labeo calbasu* in Bangladesh (Wahab et al., 1999). In Malawi two indigenous species *Labeo mesops* and *Labeo cylindricus* have been tested for small-scale aquaculture (Hiroyuki et al., 1999). The present study utilized *L. victorianus* as a test species as a step towards sustainable culture of the species.

This study therefore investigated the effect of C/N ratio levels and stocking density on pond environmental quality (water quality and sediment quality) in *L. victorianus* ponds using maize flour as a carbon source.

2. Materials and methods

2.1. Experimental fish

Juveniles of *L. victorianus* were collected by using of an electro fisher from Mara river Nyanza Province, Kenya. After collection, juveniles were transferred into inert polyethylene containers with river water and transported to the farm. From this stock, juveniles ranging from 5–10 g body weight and 5.0 to 8.5 cm total length were selected and were held at densities of 10, 15 and 25 fingerlings per m^2 (SD_{10}, SD_{15} and SD_{25}), in white, 100 μm mesh size hapas. A sub-sample of juveniles in the size range of experimental individuals was taken from the stock and weighed using an electronic digital balance (make: Orion; precision: 1 mg) to determine the initial live body weight which varied from 4.75 to 9.45 g. Differences in total length and body weight of these juveniles were analyzed with a non-parametric analysis of variance (Kruskal and Wallis, 1952). No significant differences were found in the initial total length and body weight of the fish stocked in the different experimental units (Kruskal–Wallis; $H = 0.62$, $P = 0.73$).

2.2. Experimental design

The experiment was conducted in a 3 × 2 factorial design, with three levels of fish density in hapas as first factor and C/N ratios with two levels as second factor; C/N ratio 10 and 20. Six, 150 m^2, average depth 1 m earthen ponds were used in this study. Ponds were limed at 2000 kg ha^{-1} with agricultural lime prior to filling. Eighteen hapas made from 100 μm mesh cloth, and 1 m^3 volume (1 × 1 × 1 m) were attached to wooden poles in 6 ponds (3 hapas per pond) so that the hapa bottom was 10 cm above the pond bottom. The tops of the hapas were covered with hapa cloth to prevent predation and fish escape. The 100 μm mesh ensured that feed spillage was minimal. To replace water loss due to seepage and evaporation, water was added to the ponds on a weekly basis.

2.3. Experimental site and pond management

The experiment was carried out at National Aquaculture Research Development and Training Centre (NARDTC) Sagana for a period of 72 days. All hapas received a 30% crude protein diet with a C/N ratio of 10 at 3% body weight per day. Feed was administered twice daily at 09:00 and 03:00 o'clock. The C/N 20 ponds received additional locally purchased maize (*Zea mays*) flour, at 1.14 kg for each 1 kg of 30% protein feed. The pre-weighed maize flour starch was mixed in a beaker with hapa water and uniformly distributed over the hapa surface directly after the feed application at 09:00 h. The diet proximate composition is detailed in Table 1. The daily feed quantity was adjusted biweekly after sampling.

2.4. Growth analysis and estimation of yield parameters

Fish growth calculations involved computation of mean weight (g) and their standard deviations (±SD) for fish samples from each treatment and stocking density at each sampling occasion. Graphical plots of mean weights against time were used to visualize growth. At the end of the experiment all fish were harvested and weighed up to the nearest 0.1 g. Specific growth rate (% body weight day^{-1}) was calculated using the formula, SGR $(\ln WT_F - \ln WT_I) * 100 / T$ where WT_F = average final fish weight (g), WT_I = average initial fish weight (g), T = duration of the experiment (days). Feed conversion ratio (FCR) and net yields were calculated as follows:

$$\text{FCR} = \text{feed applied (dry weight)/live weight gain.}$$

$$\text{Net yield} = \text{Total biomass at harvest–total biomass at stocking.}$$

Geometric mean body weight (Wg) was calculated to determine the estimate for the body weight of the fish at the middle of the experiment period. Calculated as $Wg = {}_e((\ln WT_F + \ln WT_I)/2)$.

Metabolic growth (RGR_m) was calculated to determine the growth achieved by the fish after utilizing the available food to generate energy for metabolism during the experiment period. Calculated as; $RGR_m = (WT_F - WT_I)\, W_g^{0.8} / T$.

Metabolic feeding rate was calculated to determine the daily feeding rate of the experimental fish. Calculated as (g feed/day/kg^0.8).

2.5. Determination of water quality parameters

Water samples were collected using a horizontal water sampler from inside the hapa and from the pond. Samples were pooled before analysis. Water quality parameters, surface and bottom temperature (Celsius thermometer), surface and bottom dissolved oxygen (YSI digital DO meter, model 58) and pH (Corning 445 pH meter) were monitored *in situ* at sunrise (07:00 h) and sunset (18:00 h) on a weekly basis. Transparency (Secchi disk) was recorded weekly at 10:00 h. Before nutrient analysis, water samples were filtered through microfiber glass filter paper (Whatman GF/C), using a vacuum pressure air pump. Total alkalinity (titrimetric method) and NO_2–N, NO_3–N, NH_3–N and PO_4–P concentrations according to standard methods described in American Public Health Association (APHA, American Water Works Association and Water Pollution Control Federation, 1995) cross-referenced to Boyd and Tucker (1992). The filter paper was kept in a test tube containing 10 mL of 90% acetone, ground with a glass rod and preserved in a refrigerator for 24 h. Later, chlorophyll-*a* was determined using a spectrophotometer (Milton Roy Spectronic,

Table 1
Proximate composition of the prepared feed and maize flour. The percentages are given on a wet weight basis.

Proximate composition %	Feed ingredients			Treatment diets	
Overall composition %	Rice bran	Cotton seed cake	Fresh water shrimps	Prepared feed	Maize floor
Crude protein	7	35	63.3	29.5	7.71
Crude lipid	4.2	10.5	1.3	7.2	4.42
Crude fiber	30.9	25	5	5.1	5.4
Ash content	22.9	63	22.8	13.2	1.52
Dry matter	92.3	89.4	87.7	87.4	88.3
Nitrogen free extacts	35	19.2	6.7	32.4	69.6